sample. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count two times greater than that used in such immunogenicity test.

[50 FR 23795, June 6, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.68 Pasteurella Haemolytica Vaccine, Bovine.

Pasteurella Haemolytica Vaccine, Bovine, shall be prepared as a desiccated live culture bacterial vaccine of an avirulent or modified strain of *Pasteurella haemolytica*, identified as serotype 1. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The immunogenicity of a selected bacterial count from the lot of Master Seed shall be established as follows:

(1) Fifteen *Pasteurella haemolytica* susceptible calves shall be used as test animals (10 vaccinates and 5 controls) for each route of administration recommended on the label.

(2) An arithmetic mean count of the colony forming units from vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 10 calves to be used as vaccinates shall be injected as recommended on the label with a predetermined quantity of vaccine bacteria. The five control calves shall be held separately from the vaccinates. To confirm the dosage calculation, five replicate titrations on a sample of the bacterial vaccine used. Only plates containing between 30 and 300 colonies shall be considered a valid test.

(3) The vaccinates and controls shall be examined and their average body temperature determined prior to challenge. Fourteen to twenty-one days post vaccination, the vaccinates and controls shall each be challenged by the respiratory route with a (virulent) pneumonia producing *Pasteurella haemolytica* culture and observed for 4 to 7 days. The challenge culture and instructions for preparation for use shall be furnished or approved by the Animal and Plant Health Inspection Service.

(4) A satisfactory challenge shall be evidenced in the controls by progression of clinical signs consistent with respiratory system infection following challenge, including but not limited to lacrimation, mucoid nasal exudates, expiratory dyspnea, tachypnea, pulmonary rales, and cough possibly terminating in death; moribundity, depression with anorexia, diarrhea with substantial weight loss; or any combination of these symptoms.

(5) Lung lesion response to challenge will be assessed in all calves. Lung lesions will be assessed at necropsy in calves that succumb to challenge. Surviving calves will be euthanized on day 4 to 7 following challenge and lung lesions assessed at necropsy. Lung lesion scores will be used in the assessment of the response to challenge exposure. If a significant difference in lung lesion scores cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed is unsatisfactory.

(6) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest: *Provided*, that, at least four of five vaccinates and four of five controls shall meet the criteria prescribed in paragraphs (b)(4) and (b)(5) of this section.

(7) An Outline of Production change must be made before authority for use of a new lot of Master Seed is granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §§113.8 and 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory

by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety in calves as provided in §§ 113.41(a) and 113.41(b) except, that the equivalent of two doses of vaccine shall be used and administered in the manner recommended on the label.

(2) *Bacterial count requirements.* Final container samples of completed product shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each serial and subserial. Each sample shall be rehydrated with accompanying sterile diluent to the volume indicated on the label. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count at least two times greater than that used in the immunogenicity test.

[55 FR 35559, Aug. 31, 1990]

§ 113.69 Pasteurella Multocida Vaccine, Bovine.

Pasteurella Multocida Vaccine, Bovine, shall be prepared as a desiccated live culture bacterial vaccine of an avirulent or modified strain of *Pasteurella multocida*, of bovine origin. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The immunogenicity of a selected bacterial count from the lot of Master Seed shall be established as follows:

(1) Fifteen *Pasteurella multocida* susceptible calves shall be used as test animals (10 vaccinates and 5 controls) for each route of administration recommended on the label.

(2) An arithmetic mean count of the colony forming units from vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 10 calves to be used as vaccinates shall be injected as recommended on the label with a predetermined quantity of vaccine bacteria. The five control calves shall be held separately from the vaccinates. To confirm the dosage calculation, arithmetic mean count shall be established by conducting five replicate titrations on a sample of the bacterial vaccine used. Only plates containing between 30 and 300 colonies shall be considered a valid test.

(3) The vaccinates and controls shall be examined and their average body temperature determined prior to challenge. Fourteen to twenty-one days post vaccination, the vaccinates and controls shall each be challenged by the respiratory route with a (virulent) pneumonia producing *Pasteurella multocida* culture and observed for 4 to 10 days. The challenge culture and instructions for preparation for use shall be furnished or approved by the Animal and Plant Health Inspection Service.

(4) A satisfactory challenge shall be evidenced in the controls by progression of clinical signs consistent with respiratory system infection following challenge, including but not limited to acute illness with higher body temperature and respiration rate, lacrimation, mucoid nasal exudate, expiratory dyspnea, tachypnea, pulmonary rales, and cough, possibly terminating in death; moribundity, depression with anorexia; diarrhea with substantial weight loss; or any combination of these symptoms.

(5) Lung lesion response to challenge will be assessed in all calves. Lung lesions will be assessed at necropsy in calves that succumb to challenge. Surviving calves will be euthanized on day 4 to 10 following challenge and lung lesions assessed at necropsy. Lung lesion scores will be used in the assessment of the response to challenge exposure. If a significant difference in lung lesion scores cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and